THE INFLUENCE OF HYDROGEN ION CONCENTRATION ON THE FERTILIZATION PROCESS IN *ARBACIA, ASTERIAS* AND *CHÆTOPTERUS* EGGS.

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In a previous communication we pointed out that when *Asterias* and *Arbacia* eggs were inseminated in CO_2-free sea water of varying H-ion concentration, fertilization failed to occur in solutions more acid than pH 6.6 to 7.0. This block to fertilization appeared to be perfectly reversible, since eggs which did not fertilize in solutions on the acid side of the block could be fertilized when returned to solutions of greater alkalinity (1). Loeb (2) has observed a similar block to fertilization in artificial salt solutions. He found that *Arbacia* and *S. purpuratus* eggs were not fertilized in a neutral mixture of $NaCl + MgCl_2$ in the proportion in which these salts exist in sea water. These eggs were fertilized, however, if NaOH, NH_4OH, benzylamine, butylamine or $NaHCO_3$ were added to the $NaCl + MgCl_2$ mixture. The addition of $CaCl_2$ to the $NaCl + MgCl_2$ mixture similarly made fertilization possible. The addition of NaOH or $CaCl_2$ to a $NaCl + KCl$ mixture did not permit fertilization of all eggs, but when both NaOH and $CaCl_2$ were added to a $NaCl + KCl$ mixture as a rule all the eggs fertilized and began to divide. Since cross fertilization can be effected between *Asterias* sperm and *S. purpuratus* eggs by the addition of NaOH or $CaCl_2$ to normal sea water, Loeb concluded that the act of diminishing the alkalinity of the solution or of depriving it of $CaCl_2$ established the same reversible block to the entrance of the homologous sperm as exists for the entrance of the sperm of *Asterias* into *S. purpuratus* eggs in normal sea water. Leob's experiments involve the change of several variables at once, however, and it cannot be determined from them to what extent the reaction of the external medium *per se* influences the fertilization of eggs by sperm of the same species.

Further examination of the block to fertilization which is created when the H-ion concentration of sea water is increased to a critical point has convinced us of its physiological significance, and we have extended our observations to include the effects of increasing alkalinity on the fertilization of *Arbacia* and *Asterias* eggs, and the effects of acid and alkaline sea water on the fertilization of the eggs of *Chætopterus pergamentaceous.*

The Acid Block to Fertilization.

CO_2-free sea water solutions were prepared as described in a previous paper (3). Our experiments on fertilization were performed as follows: A drop of concentrated egg suspension was added to 50 or 100 cc. of each of the pH solutions, and a drop of sperm suspension was added to about 5 cc. of the pH solutions. After an interval of 3 to 5 minutes the sperm and eggs were mixed and thoroughly agitated. (No precautions were taken to remove body fluids which might be present around the eggs, other than the routine washing which they were always given in preparing them for any experiment.) Subsequently the proportion of fertilized eggs in each dish was carefully determined. It makes no difference whether the counts are made 10 minutes or several hours after insemination because every egg that is going to fertilize will lift a membrane within the normal time of 3 to 5 minutes. It has been our custom in performing experiments of this kind to remove samples from the pH solutions 10 or 15 minutes after insemination and return them to sea water with fresh sperm to make sure that the eggs had not been irreversibly modified by the action of the pH solutions or by contact with sperm in these solutions. It may be said that this procedure has one invariable result; if the exposure is below that required for the acid to injure the egg, then every egg which is not fertilized on the acid side of the block will fertilize when returned to sea water with fresh sperm.

The influence of H-ion concentration on the fertilization of *Arbacia* and *Asterias* is illustrated in Figs. 1 and 2. The solid line in each figure indicates the range within which fertilization

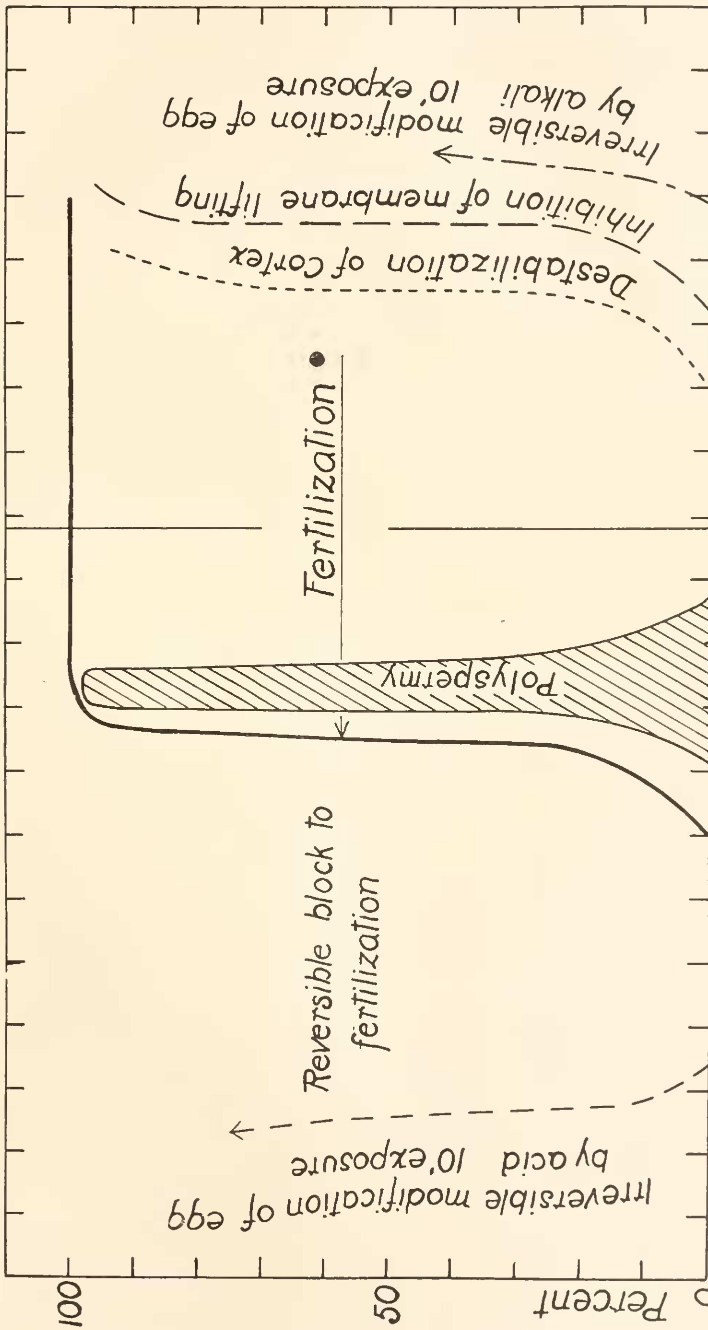

FIG. 1. The influence of H-ion concentration on the fertilization of *Arbacia* eggs.

occurs. The cessation of fertilization with increasing acidity is very abrupt, but the critical H-ion concentration may be most accurately indicated by the pH at which only 50 per cent. of the eggs fertilize. This critical H-ion concentration is pH 6.8 for *Arbacia* eggs, and 7.0 for *Asterias* eggs. In solutions slightly more alkaline than these, all the eggs fertilize; and in solutions slightly more

Table I.

A. Acid Block to Fertilization in *Asterias*.

Per Cent. of Eggs Fertilized.

pH									Date.
6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	
0		0		50		100		100	Conclusions 1921.
0		1		42		100			6–30–22
0		0		21		85			7– 6–22
0		0		0		4		76	5–29–23
0		0	1	5	5	40		72	5–30–23
0	0	0	0	47	60	85		93	8–31–23
0	0	0	0	0		82		100	8–31–23
0	0	0	0	60		100		100	8–31–23
0	0	0	10	6		21		72	9– 2–23
0	0	0	14	11		22		90	9– 3–23
0	0	0	40	40		100		100	9– 3–23

B. Acid Block to Fertilization in *Arbacia*.

Per Cent. of Eggs Fertilized.

pH									Date.
6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	
0		50		100		100		100	Results of 1921.
2		47		98		98		97	6–30–22
0	0	100	100	100	100	100		100	7–20–23
0	0	30	20	75	100				9–21–23
2	7	28	24	95	100	100	100		9–22–23
7	29	100	100	100	100	100	100	100	9–22–23
0	0	30	20	75	100	100	100	100	9–22–23

C. Comparison of Acid Block in *Asterias* and *Arbacia* in Same pH Solutions. 9–21–23.

Per Cent. of Eggs Fertilized.

	pH								
	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4
Asterias.	0	0	0	1	30	100	100	100	100
Arbacia.	5	0	30	70	100	100	100	100	100

acid, none of the eggs fertilize. We have previously shown that the unfertilized eggs of these species are uninjured by short exposures to the solutions in which fertilization does not occur (3); and that normally fertilized eggs will develop with normal velocity at H-ion concentrations much greater than those at which the block to fertilization occurs (4).

The constancy of this acid block to fertilization is very marked. To illustrate this a few experiments have been given below. Table I. contains a résumé of experiments performed in 1921, 1922 and 1923 on the fertilization of *Asterias* and *Arbacia* eggs. Despite the probability of variable conditions in these experiments, the point at which 50 per cent. of the eggs fertilized remained constant to $\pm$ 0.2 pH.

There are at least two factors which might be expected to shift the block one direction or another; first, the length of time which the eggs or sperm have remained in the acid solution, and second, the relative quantity of sperm used for insemination. Examination of the first factor has shown that the equilibrium between the pH solution and the egg (or sperm) is reached with astonishing rapidity. This can be illustrated by first adding the sperm to the pH solution and then adding to the resulting sperm suspension a drop of eggs suspended in sea water. Under these conditions one would expect that the time required for the egg cortex to come to chemical equilibrium with the pH solution would be long enough to permit many more eggs to be reached by sperm and fertilized than would be the case if the eggs were allowed to come to equilibrium with the solution before adding the sperm. The results of experiments of this kind with *Asterias* eggs are given in Table II. Converse experiments were simultaneously performed; the eggs were added to the pH solution first and after 5 minutes a drop of comparatively concentrated sperm suspended in sea water was added to these eggs. When the experiment is performed as first described, the block appears at the same pH as when both eggs and sperm are at equilibrium with the pH solutions before insemination. When the experiment is reversed, the block is shifted slightly towards the alkaline side. This indicates that the essential equilibrium underlying the block involves the egg cortex rather than the sperm. The difference is hardly great enough to be significant, though the results do show very definitely that chemical equilibrium between either eggs or sperm and the pH solutions is reached in less time than is required for sperm to reach the eggs and fertilize them.

TABLE II.

A. EFFECT OF EXPOSING *Asterias* SPERM TO PH SOLUTIONS FOR 5 MINUTES BEFORE ADDING *Asterias* EGGS IN SEA WATER. 8–31–23.

Per Cent. Eggs Fertilized.

pH									
6.2	6.6	6.8	6.9	7.0	7.1	7.2	7.6	8.15	
0	0	0	6	8	81	85	90	83	(Exp. 1)
0	0	0	0	47	85	90	89	97	(Exp. 2)

B. EFFECT OF EXPOSING *Asterias* EGGS TO PH SOLUTIONS FOR 5 MINUTES BEFORE ADDING *Asterias* SPERM IN SEA WATER. 8–31–23.

Per Cent. Eggs Fertilized.

pH								
6.2	6.6	6.8	6.9	7.0	7.1	7.2	7.6	
0	0	0	0	0	49	82	100	(Exp. 1)
0	0	0	0	2	63	95	100	(Exp. 2)

One of several experiments testing the influence of varying quantities of sperm in shifting the limits of fertilization is given in Table III. The eggs were placed in the pH solutions and 5 minutes later the sperm, diluted with the pH solutions, were added.

TABLE III.

INFLUENCE OF QUANTITY OF SPERM ON ACID BLOCK IN *Asterias.*

Per Cent. Eggs Fertilized.

pH										Quantity of sperm added to 25 cc. pH sol.
6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	8.15	
0	0	0	50	100	95	95	100	100	100	1 cc. 1–20
0	0	0	0	35	80	95	100	100	100	1 cc. 1–200
0	0	0	0	0	20	85	87	95	90	1 cc. 1–2,000
0	0	0	0	1	40	45	25	35	30	1 cc. 1–20,000

The smallest quantity of sperm was insufficient to fertilize all the eggs even in sea water, and the largest quantity gave a distinctly opalescent suspension; yet the block did not shift beyond the limits pH 6.9 to 7.1. In general, increasing the quantity of sperm used in insemination increases the proportion of eggs fertilized in the acid solutions, but the shift to the acid side is not so great as would be expected if the failure to fertilize in the acid solutions were attributable to an impairment of the sperm. Rather the slight magnitude of this shift favors the belief that the block is due to an alteration of the properties of the egg.

It may be stated here that unless the sperm are injured or attenuated by the toxic action of egg secretions, all eggs which fertilize in the pH solutions develop normally, indicating that the fertilization reaction when once initiated in the neighborhood of the block, is completed without impairment.

The Acid Block in *Chætopterus*.

The determination of the acid block to fertilization in *Chætopterus* was made in the same manner as in *Asterias* and *Arbacia*. The egg sacks were cut in sea water and the eggs liberated from the ovaries by teasing these to pieces. The ovary fragments were removed by straining through cheese cloth, and when the eggs had maturated they were concentrated by centrifuging. A drop of the concentrated egg suspension was added to 50 cc. of the pH solution; after 5 minutes the sperm, previously diluted with the pH solutions, were added and the mixture agitated. The per cent. of fertilized eggs was determined by counting the dividing eggs one and a half to four hours after insemination.

The scarcity of material made it impossible to get more than a half dozen determinations; of these, two were discarded since only a small proportion of the eggs were fertilized in sea water. The remaining four indicated that the block appeared between pH 7.0 and 7.3, and from the two most satisfactory experiments the block was tentatively set at pH 7.1.

The acid (pH 5.8) activation of the *Chætopterus* egg, with the consequent temporary block to fertilization, has been discussed in a previous paper (3). This block, which is most effectually established by short exposures to pH 5.2 to 6.4, was tentatively ascribed to cortical changes which tend to persist after the eggs have been removed from the acid solutions, and returned to sea water. It is in no sense comparable to the physiological block occurring at pH 7.1; the latter is perfectly and instantly reversible, as in *Asterias* and *Arbacia,* disappearing as soon as the eggs are returned to a more alkaline solution.

The Influence of Alkali on the Fertilization Reaction.

Frank Lillie (5) has observed that the addition of alkali to the sea water in which insemination occurs increases the incidence of fertilization in *Asterias* and *Arbacia* eggs of poor quality. We have confirmed this in *Asterias* and *Arbacia* and found that it is equally true for *Chætopterus.* This effect of a slight increase in alkalinity in aiding fertilization may be due to action on the sperm but it seems more probable that both the eggs and the sperm are affected. The changes which culminate in increased fluidity of the egg cortex in alkaline sea water (3) are no doubt preceded by enhanced physiological reactivity.

Apart from this stimulating action of alkali, which is not apparent in eggs of the best quality, fertilization in *Asterias* and *Arbacia* proceeds unimpaired from pH 8.15 to 9.6. With further increases in alkalinity, eggs appear in increasing numbers which have either tight or incompletely formed fertilization membranes; and at pH 10.2 the eggs have no demonstrable membranes at all. When returned to sea water after a 3 to 5 minute exposure to pH 10.0, fertilization membranes will form on most of the previously unmembraned eggs. It was concluded that these eggs were fertilized while in the alkaline solution, since the supernatant sperm carried over from the alkaline solution are incapable of fertilizing fresh eggs. Longer exposures injure the eggs to such an extent that membranes do not form on them when they are returned to sea water. The H-ion concentrations which prevent membrane elevation (and which destabilize the cortex of the unfertilized egg (4)) are shown in Figs. 1 and 2 by the dotted lines at the extreme right.

This alkaline injury is more rapid in *Asterias* than in *Arbacia.* In the latter case the eggs will divide imperfectly if returned to sea water after a 5 to 10 minute exposure to the alkaline solution. In *Asterias* the inhibition of membrane elevation is rapidly followed by a more profound injury which completely stops development. Such eggs can not be fertilized by fresh sperm in sea water.

These facts all indicate that under increased alkalinity union of the egg and sperm still occurs; but if the increased alkalinity

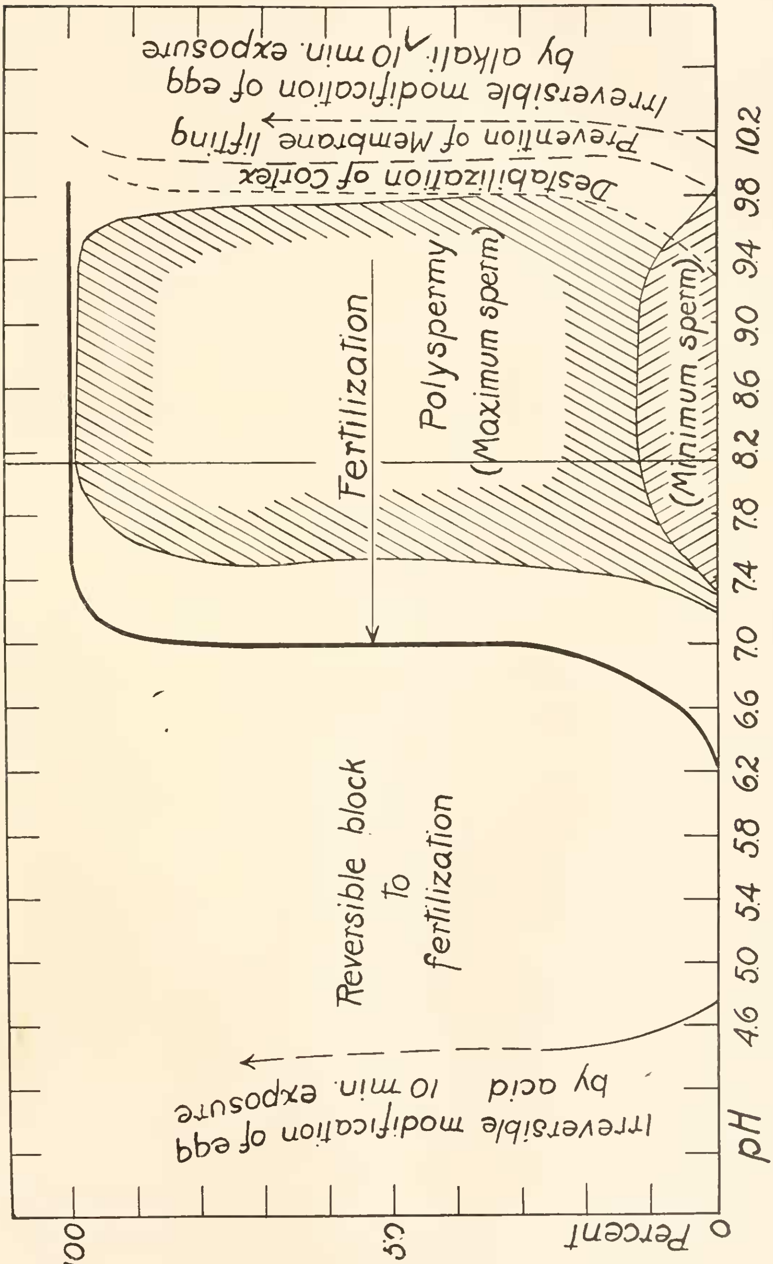

FIG. 2. The influence of H-ion concentration on the fertilization of *Asterias* eggs.

does not actually introduce some abnormality into this initial event, it impairs subsequent events of the fertilization process to such a degree as to prevent normal development. There is apparently no reversible block created by alkali corresponding to that created by acid, where the fertilization reaction proceeds in an all-or-none fashion. This conclusion is supported by the extremely rapid injury of the eggs and sperm if separately exposed to the alkaline solutions (pH 10.0), which prevent normal fertilization, and the complete absence of such injury in acid solutions (pH 6.8 to 7.0) which have a similar effect.

Polyspermy.

In the case of *Arbacia* eggs there is a very narrow range in H-ion concentration in which the incidence of polyspermy is unusually high. This range is approximately defined in Figure 1 by the heavily shaded portion; the maximum of polyspermy is close to pH 7.2. Though the incidence of polyspermy at all H-ion concentrations increases with increasing age or staleing of the eggs, yet within this narrow range, centering at pH 7.2, practically all the eggs will be polyspermic even when they are fresh and when the incidence of polyspermy is nearly zero from pH 7.4 to 9.8.

In *Asterias,* polyspermy shows no marked maximum at any H-ion concentration but occurs more or less uniformly from pH 8.5 to 9.5 (Fig. 2). When excessive quantities of sperm are used in insemination, nearly all the eggs may be polyspermic from 8.15 to 9.6. It is perhaps significant that the polyspermy curve, even though extremely broad, is limited on the alkaline side; for the incidence of polyspermy decreases appreciably before the alkalinity is sufficient to inhibit fertilization, indicating that in its general nature the underlying mechanism in *Asterias* is similar to that in *Arbacia.*

We did not have the opportunity to make similar observations on polyspermy in *Chætopterus.* Such data as we have indicate that there is, as in *Arbacia,* a comparatively narrow region in which polyspermy predominates (about pH 9.5).

Reaction of Sperm with Immature *Asterias* Eggs.

When immature *Asterias* eggs are inseminated in sea water, several sperm usually enter each egg before the fertilization membrane is formed. Subsequently the germinal vesicle breaks down and the cytoplasm acquires a mottled appearance, each sperm being the focus of a localized cytolytic process. Such prematurely fertilized eggs never attempt to divide. If sperm are added to immature eggs at various H-ion concentrations, a block appears at the same point as in the fertilization of the mature egg, viz., pH 7.0. On the alkaline side of this point the sperm enter the eggs, causing membrane elevation and the changes described above. On the acid side the sperm do not react with the eggs in any way; in the course of time, a varying proportion of these unfertilized eggs will maturate, depending on the H-ion concentration, and these, if they are returned to sea water and inseminated, will fertilize and develop normally.

Summary.

When *Arbacia, Asterias* and *Chætopterus* eggs are inseminated in CO_2-free sea water of varying H-ion concentration, a block to fertilization appears at a H-ion concentration which is constant, and apparently characteristic for each species. If the block is defined by the H-ion concentration at which 50 per cent. of the eggs fertilize, these H-ion concentrations are: *Arbacia,* pH 6.8; *Asterias,* pH 7.0; and *Chætopterus,* pH 7.1.

This block to fertilization is complete, in that eggs either fertilize and develop normally, or do not fertilize at all; and it is perfectly reversible, in that eggs which do not fertilize on the acid side of the block will fertilize immediately if they are returned to solutions on the alkaline side of the block and inseminated with fresh sperm.

In sea water more alkaline than pH 9.8 to 10.0 the fertilization process in both *Arbacia* and *Asterias* eggs is either incomplete or impaired. Apparently there is no alkaline block to fertilization corresponding in its complete reversibility to the block which appears around neutrality.

In *Arbacia* there is an increased incidence of polyspermy within a very narrow range centering at pH 7.2, indicating some critical

condition in the mechanism of fertilization at this H-ion concentration. In *Asterias* polyspermy occurs more or less uniformly over a wide range extending from pH 7.2 to 9.8.

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